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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 35/74	A1	(11) International Publication Number: WO 96/26732
		(43) International Publication Date: 6 September 1996 (06.09.96)

(21) International Application Number: PCT/EP96/00558

(22) International Filing Date: 9 February 1996 (09.02.96)

(30) Priority Data:
MI95A000378 28 February 1995 (28.02.95) IT

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(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS OF LACTIC ACID BACTERIA AND SACCHAROMYCES LYSATES AND THE THERAPEUTICAL USE THEREOF

(57) Abstract

Combinations of lactic acid bacteria and Saccharomyces lysates are described having immunostimulating activity, the use thereof in human and veterinary medicine and pharmaceutical compositions containing them.

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COMPOSITIONS OF LACTIC ACID BACTERIA AND SACCHAROMYCES LYSATES AND THE THERAPEUTICAL USE THEREOF

The present invention relates to combinations of lactic acid bacteria and Saccharomyces lysates and/or glucans, the use thereof as immunostimulating agents and pharmaceutical compositions containing them.

Technological background

Lactic Acid Bacteria, hereinafter referred to as LAB, which comprise, inter alia, Lactobacilli (among which Acidophylus, Bulgaricus, Delbreukii, Fermentum, Casei), Streptococci (among them, Thermophylus), Bifidobacteria (Longum, Biphydus, Pullorum, inter alia) and sporogenous lactobacilli, such as Lactobacillus sporogenes, have been used for a long time in pharmaceutical, dietetic or food products and administered to restore bacteria flora in dysmicrobisms of various nature, for example due to antibiotic therapies or to diseases of the gastro-intestinal tract (Bottazzi, Zacconi, Sarra, Probiotica con Batteri Lattici, Centro Sperimentale del Latte, Milan, Italy). LAB, besides being a direct source of Vitamins of the groups B, D, K, and the like, act through a mechanism of competitive antagonism against microorganisms, pathogenic and/or opportunists, which, in particular conditions, have affected the normal intestinal bacterial flora (Bottazzi, Zacconi, Sarra, Probiotica con Batteri Lattici, Centro Sperimentale del Latte, Milan, Italy).

Bacteria are known to have (Sharon et Lis,

Carbohydrates in Cell recognition - Sci. Am.-Jan. 93) a cell wall characterized by the presence of specific polysaccharides which allow for the bacterium itself to adhere to the intestinal epythelium, the latter being characterized in turn by analogue polysaccharide receptors. The presence of said receptor sites contributes to establish the antagonism among the various bacteria, when the micro-climatic conditions vary (pH, redox potential).

The polysaccharides present on the cell walls are of different nature, but polysaccharides deriving from mannan, characterized by α1-6, 1-2 and 1-3 bonds, from glucans (β1-3 polyglucose) are always present.

It is known that mannans (Technical Publication n. 113 Alltech Inc. Nicholasville, KY, U.S.A.) can be used to favourably change the intestinal ecosystem by competitively inhibiting the adhesion sites which could be used as adhesion and colonization points by pathogenic microorganisms. Fructans and glucans (polyfructose and polyglucose, respectively) were employed as an alternative to mannans, with the same purposes. Fructans turned out to be easily fermentable by a great number of microorganisms which on the contrary cannot hydrolyse glucans (only some species of microorganisms can hydrolyse and ferment glucans).

Moreover, mannans are known (Technical Publication n. 113 Alltech Inc. Nicholasville, KY, U.S.A.) to have a strong stimulating action in the growth of such LAB as Bifidobacteria, Lactic acid bacteria and Streptococci.

Disclosure of the invention

It has surprisingly been found that, upon

administration of a combination of LAB and Saccharomyces lysates containing glucans for the treatment of intestinal dysmicrobiosis, a significant increase in all of the blood elements, both formed and non formed, involved in the non-specific immune response (macrophages, lymphocytes etc.) takes place.

Therefore, it is an object of the present invention a combination comprising:

a) lactic acid bacteria;

b) Saccharomyces lysates; and optionally

c) glucans,

being it possible to replace component b) with component c).

It is another object of the present invention the use of the combination as an immunostimulating agent.

It is still another object of the present invention the use of the combination as an active ingredient, optionally in the presence of other pharmacological agents, for the manufacture of a medicament useful to improve immune defences in man and in animals.

A further object of the present invention is provided by pharmaceutical compositions containing the combination as the active ingredient.

Detailed disclosure of the invention

According to the present invention, lactic acid bacteria or LAB, as herein meant, comprise lactic acid bacteria in a broad sense. Examples of LABs are Lactobacillus acidophylus, L. bulgaricus, L. delbreukii, L. casei, L. Fermentum, the Streptococcus genus, for example S. thermophylus, Bifidobacteria, such as B. longum, B. biphydus, B. pullorum, the sporogenous genus,

for example Lactobacillus sporogenes.

The Saccharomyces lysates can be obtained by methods known to those skilled in the art, see for instance J. A. Reynolds et al., Infection and Immunity, Oct. 1980, vol. 30, n. 1, p. 51-57 and the literature therein cited.

Glucans of different purity can be obtained by known methods and are also commercially available.

According to the present invention, the ratio of component a) to component b), the latter optionally in admixture with component c), or of component a) to component c), ranges from 1:1,000 to 1:0.001.

According to a first preferred embodiment of the present invention, the lysate is obtained from Saccharomyces cerevisiae.

According to another preferred embodiment of the present invention, the LAB is selected from the group consisting of L. acidophylus, L. bulgaricus, or a combination thereof, L. thermophylus.

The immunostimulating action of glucan being known (J. A. Reynolds et al. - Glucan-Induced Enhancement of Host Resistance to Selected Infectious Diseases, Infect. et Immun. 30, 1, 51-57 - Oct. 1980) (Kokoshis et al. - Increased Resistance to Staphylococcus aureus Infection and Enhancement in Serum Lysozyme Activity by Glucan - Scie. 199, March 24, 1978), the action on the immune system of the single components of the combination (LAB and Saccharomyces cerevisiae lysates) was evaluated.

Thus, the immunostimulating action induced by the combination of LAB and Saccharomyces cerevisiae lysate and/or glucans was evidenced to be substantially higher

than that found in the subjects treated with only Saccharomyces lysates (glucans) ~> 15%) and with LAB only (> 70%). The actions exerted by Saccharomyces cerevisiae lysate and that of glucans were not substantially different.

Further controls proved that, in the treated subjects, the immune responses from subjects treated with only glucan and/or Saccharomyces lysate are statistically different from those obtained in subjects treated with the combinations of the two products. In fact, an increase in macrophages, as well as the presence of lymphocytes joined to an increase in lysozyme production moreover shown by the increase in the development of the cell endoplasmic reticulum.

The oral administration of a combination of Saccharomyces cerevisiae lysates and/or glucans with LAB proved to be a simple, effective means to improve immune defenses in man and animals.

Such an administration can be carried out by use of pharmaceutical compositions containing the combination of the invention in admixture with conventional carriers and excipients. The compositions can be solid, such as tablets (prompt-release or controlled-released ones), coated tablets (film-coated, gastro-resistant, controlled-release, sugar-coated pills), effervescent or fast-dissolution tablets, chewable tablets, soft- and hard-gelatin capsules, sugar-coated pills; or they can be liquid, such as solutions, suspensions, syrups, elixirs, granulates for suspensions or solutions to be reconstituted.

The compositions according to the invention can be

prepared with techniques known to those skilled in the art, for example as described in "Remington's Pharmaceutical Sciences Handbook, XVII Ed., Mack Pub., N.Y., U.S.A..

The daily dosages will be established by the physician, depending on the disease to treat and the conditions of the patient (age, sex, weight).

By way of examples, the doses can range as follows:

LAB: 1 million of c.f.u./dose to 100 billion of c.f.u./dose;

Saccharomyces lysates: 0.1 mg/dose to 1 g/dose;

glucans: 0.1 mg/dose to 1 g/dose.

The daily dose can vary from 1 dose/day to 5 doses/day.

According to the present invention, the compositions and the medicaments can be used both in human and veterinary medicines.

The compositions of the invention for the improvement of the immune responses in man and animals can be used not only for therapeutic, but also for prophylactic purposes. For the latter, besides the above mentioned pharmaceutical compositions, the present invention also comprises dietetic and food compositions containing the combination described above.

The following examples further illustrate the invention.

Example 1

Capsules

One capsule contains:

Lactobacilli Acidophylus and Bulgaricus	10 billion
Saccharomyces lysates	100 mg

Lactose	500 mg
Magnesium Stearate	20 mg

Capsule coating:

Food gelatin

Titanium dioxide.

Example 2

Tablets

One tablet contains:

Lactobacilli Acidophylus and Bulgaricus	20 billion
Saccharomyces lysates	300 mg
Microcrystalline cellulose	600 mg
Talc	20 mg
Magnesium Stearate	10 mg

Example 3

Granulate sachets

One dosage unit contains:

Lactobacillus Thermophylus	10 billion
Saccharomyces lysates	100 mg
Saccharose	5,000 mg
Orange flavour	30 mg

Example 4

Vials with dosage cap

Content of dosage cap:

- Lactobacillus Sporogenes Spores	500 million
- Lactobacillus Thermophylus	500 million

Contents of vial:

- Saccharomyces C. lysates	200 mg
- Saccharose	2 g
- Methyl-p-hydroxybenzoate	0.0075 mg
- Depurated water q.s. to ml	5.

CLAIMS

1. Combinations comprising:

a) lactic acid bacteria

b) Saccharomyces lysates, and optionally

c) glucans,

being it possible to replace component b) with component c).

2. Combinations according to claim 1, wherein the lactic acid bacteria are selected from the group comprising the genera Lactobacillus, Streptococcus, Bifidobacteria and sporogenous lactobacilli.

3. Combinations according to claim 2, wherein the Lactobacillus is selected from the group consisting of Lactobacillus acidophylus, L. bulgaricus, L. delbreukii, L. casei, L. Fermentum, or combinations thereof.

4. Combinations according to claim 2, wherein the Streptococcus is S. thermophylus.

5. Combinations according to claim 2, wherein the Sporogenous is Lactobacillus Sporogenes spores.

6. A combination according to claim 2, wherein the Bifidobacterium is selected from the group consisting of B. longum, B. biphydus, B. pullorum o combinations thereof.

7. Combinations according to any one of the above claims, wherein the ratio of component a) to component b), the latter optionally in admixture with component c), or of component a) to component c), ranges from 1:1,000 to 1:0.001.

8. Combinations according to any one of the above claims, wherein the lysate is obtained from

Saccharomyces cerevisiae.

9. The use of the combinations of claims 1-8 as immunostimulating agents.

10. The use of the combinations of claims 1-8 as active ingredient for the preparation of a medicament useful for the improvement of the immune defenses for the therapeutical or prophylactic purposes in man and animals.

11. Pharmaceutical compositions containing as the active ingredient the combinations of claims 1-8 in admixture with conventional carriers and excipients.

12. Compositions according to claim 11, selected from the group consisting of prompt-release tablets, controlled-release tablets, coated tablets, sugar-coated pills, effervescent or fast-dissolution tablets, chewable tablets, soft- and hard-gelatin capsules.

13. Compositions according to claim 11, selected from the group consisting of solutions, suspensions, syrups, elixirs, granulates for suspensions or solutions to be reconstituted.

14. A pharmaceutical composition in the form of capsules according to the claims 11-12, comprising for each capsule:

Lactobacilli Acidophylus and Bulgaricus	10 billion
Saccharomyces lysates	100 mg
Lactose	500 mg
Magnesium Stearate	20 mg
Capsule coating:	
Food gelatin	
Titanium dioxide.	

15. A pharmaceutical composition in the form of tablet

according to the claims 11-12, comprising for each tablet:

Lactobacilli Acidophylus and Bulgaricus	20 billion
Saccharomyces lysates	300 mg
Microcrystalline cellulose	600 mg
Talc	20 mg
Magnesium Stearate	10 mg.

16. A pharmaceutical composition in the form of granulate according to the claims 11-12, comprising for unitary dose:

Lactobacillus Thermophylus	10 billion
Saccharomyces lysates	100 mg
Saccharose	5,000 mg
Orange flavour	30 mg.

17. A pharmaceutical composition in the form of vials with reservoir plug according to the claims 11-12, comprising:

Content of dosage cap:

- Lactobacillus Sporogenes Spores	500 million
- Lactobacillus Thermophylus	500 million

Contents of vial:

- Saccharomyces C. lysates	200 mg
- Saccharose	2 g
- Methyl-p-hydroxybenzoate	0.0075 mg
- Depurated water	q.s. to ml 5.

INTERNATIONAL SEARCH REPORT

Inv ional Application No
PCT/EP 96/00558

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	FR,A,2 718 752 (WORLD TRUST INVESTMENT) 20 October 1995 see claims 1,12 see page 5; example 1 see page 24, line 4 - line 34 ---	1-17
X	PATENT ABSTRACTS OF JAPAN vol. 17, no. 394 (C-1088) & JP,A,05 076292 (MARUTA KIYOSHI), 3 March 1993, see abstract ---	1-17
X	EP,A,0 396 744 (KABUSHIKI KAISYA ADVANCE) 14 November 1990 see claims 1,6 see page 10, line 1 - page 11, line 5 see page 12, line 3 - line 5 --- -/--	1-8, 11-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 July 1996	16.07.96

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IT,B,1 239 743 (GORKI EPIDEMIOL. MICROBIOL. INST.) 15 November 1993 see page 13, line 4 - line 14 see page 14; example 1	1-8, 11-17
Y	INFECT.IMMUN., vol. 30, no. 1, 1980, pages 51-57, XP000574740 "Glucan-induced enhancement of host resistance to selected infectious diseases" cited in the application see page 51	1-17
Y	WO,A,94 26114 (IMMUNOM TECHNOLOGIES INC.) 24 November 1994 see claim 1 see page 19, line 8 - line 13	1-17
Y	WO,A,89 11858 (WITECHLIFFE LAB.) 14 December 1989 see page 3, line 1 - line 14	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A-2718752	20-10-95	FR-A-	2718751	20-10-95
EP-A-396744	14-11-90	DE-D-	3885468	09-12-93
		DE-T-	3885468	24-02-94
		WO-A-	8900425	26-01-89
IT-B-1239743	15-11-93	NONE		
WO-A-9426114	24-11-94	NONE		
WO-A-8911858	14-12-89	AU-B-	3836489	05-01-90